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Prominent Lectin-Like Oxidized Low Density Lipoprotein (LDL) Receptor-1 (LOX-1) Expression in Atherosclerotic Lesions Is Associated with Tissue Factor Expression and Apoptosis in Hypercholesterolemic Rabbits

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Background: Despite increasing *in vitro* evidence that lectin-like oxidized low density lipoprotein (LDL) receptor-1 (LOX-1), a cell-surface receptor for oxidized LDL, is implicated in the atherogenesis and thrombus formation, its *in vivo* participation to the atherosclerotic plaque destabilization, rupture and thrombus formation remains unclear. Here, we compared the *in vivo* expression of LOX-1, with tissue factor (TF) expression and cell apoptosis, in atherosclerotic lesions of myocardial infarction-prone Watanabe heritable hyperlipidemic (WHHLMI) rabbits. Methods and Results: We prepared sixty series of cross sections in the aortic arch and the thoracic aorta from four WHHLMI rabbits. LOX-1 and TF expression, as well as apoptotic events were determined by immunohistochemical staining and TUNEL methods, respectively. LOX-1 expression was mainly observed in the macrophage-rich lipid areas of vulnerable plaque-like atheromatous lesions where TF expression and apoptotic events were prominent. LOX-1 expression was positively correlated with TF expression ($r=0.53$, $p<0.0001$), apoptotic events ($r=0.52$, $p<0.0001$) and morphological vulnerability ($r=0.63$, $p<0.0001$). Conclusions: LOX-1 expression appears to be closely associated with TF expression, apoptotic events and the morphological vulnerability, suggesting the *in vivo* involvement of LOX-1 in the destabilization and rupture of atherosclerotic lesions and the subsequent thrombus formation. The present findings in hypercholesterolemic rabbits should help advance our understanding of the pathophysiology of atherosclerosis.

Key words lectin-like oxidized low density lipoprotein receptor-1; tissue factor; apoptosis; atherosclerosis

Rupture or erosion of vulnerable atherosclerotic plaques and the subsequent formation of occlusive thrombi are currently recognized as the primary causes of acute coronary syndrome and stroke.[1] Vulnerability of atherosclerotic plaques, rather than severity of luminal stenosis, has been suggested to be the most important determinant for the onset of acute coronary syndrome.[2] Accordingly, it is of great importance to determine causative factors in the destabilization of atherosclerotic plaques and in the thrombus formation, which should help develop new therapeutic and diagnostic (imaging) agents of atherosclerosis, leading to the establishment of novel therapeutic strategies for preventing acute coronary syndromes and stroke.

To date, enhanced proinflammatory responses and the expression of matrix metalloproteinases (MMPs) have been suggested to play important roles in the destabilization of atherosclerotic plaques. Apoptosis and prothrombotic factors in atherosclerotic plaques have also been reported to be crucial factors for the plaque vulnerability and thrombus formation.[3] Apoptosis of foam cells and macrophages contributes to the formation of an acellular (cell-poor) lipid core,[4] and the apoptosis of smooth muscle cells further weaken the fibrous cap by decreasing the synthesis of extracellular matrix proteins.[5] Tissue factor (TF), a cofactor for plasma coagulation factor VIIa, which is localized in vascular cells and the lipid core within the atherosclerotic lesions, is an initiator of the coagulation cascade leading to thrombus formation after the plaque rupture *in vivo*.[3]

Lectin-like oxidized low density lipoprotein (LDL) receptor-1 (LOX-1), a type II membrane glycoprotein belonging to the C-type lectin family, acts as a cell-surface receptor for oxidized LDL (Ox-LDL) and mediates several biological effects of Ox-LDL.[6] Recent studies with cultured cells suggest that LOX-1 may play several important roles in destabilization of atherosclerotic plaques, inducing expression of adhesion molecules and chemokines for monocytes,[7] transformation of macrophages into foam cells,[8,9] apoptosis of smooth muscle cells[10,11] and the degradation of extracellular matrix proteins by induction of matrix metalloproteinases.[12] Several studies with cultured cells also showed that Ox-LDL, through LOX-1, also triggers the CD40/CD40L signaling pathway,[13,14] which then induce TF expression.[15] These biological effects mediated by Ox-LDL-LOX-1 interactions may enlarge the lipid core, weaken the fibromuscular cap, and induce proinflammatory responses, resulting in destabilization of the atherosclerotic plaques and thrombus formation.

The actual contribution of LOX-1 to the plaque vulnerability and thrombus formation *in vivo*, however, remains unclear. In this context, we recently demonstrated that LOX-1 expression is related to MMP-9 expression and morphological plaque vulnerability using a rabbit model of spontaneous atherosclerosis,[16] myocardial infarction-prone Watanabe her-

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itable hyperlipidemic (WHHLMI) rabbits (previous strain: Watanabe heritable hyperlipidemic (WHHL) rabbits). The use of WHHLMI rabbits could be effective for investigating causative factors in the destabilization of atherosclerotic plaques, as the histological characteristics of their atherosclerotic lesions have been reported to be similar to those of humans.[17,18] Thus, in the present study, we compared LOX-1 expression with TF expression and apoptosis in atherosclerotic lesions of WHHLMI rabbits, in order to further characterize the roles of LOX-1 to the plaque vulnerability and thrombus formation *in vivo*.

MATERIALS AND METHODS

Animals Four WHHLMI rabbits (female, 12.6±0.8 months old: 3.7±0.3 kg body weight) bred at Kobe University were used in the present study. The rabbits were fed standard rabbit chow (type CR-3; Clea Japan Inc., Tokyo, Japan: 120 g/d) and were given water *ad libitum*. All experimental procedures were approved by the Kyoto University Animal Care Committee.

Preparation of Histological Sections Rabbits were sacrificed with an overdose of sodium pentobarbital (Nembutal, intravenously, Dainippon Sumitomo Pharma, Osaka, Japan). The aortic arch and thoracic aorta were cut into 6 and 9 segments, respectively. Each segment was immediately fixed in a solution containing L-(+)-lysine hydrochloride (75 mmol/l) and 4% paraformaldehyde in phosphate buffer (37.5 mmol/l; pH 7.4), and embedded in paraffin. Consecutive 5-μm thick slices were prepared at the center of each segment.

Histological Analysis Serial sections were subjected to immunostaininig for LOX-1, TF and cell type marker antigens, as well as HE and Azan–Mallory staining. A monoclonal antibody for rabbit LOX-1 (mouse IgG) was established by a standard hybridoma technique.[16] Its specificity was confirmed in CHO cells stably expressing rabbit LOX-1 (data not shown). A monoclonal antibody for rabbit TF (mouse IgG) was obtained from American Diagnostica (Stanford, CA, U.S.A.). Monoclonal antibodies for a rabbit macrophage-specific antigen (RAM-11, mouse IgG) and smooth muscle actin (1A4, mouse IgG) were obtained from Dako Corp., Santa Barbara, CA, U.S.A. Immunohistochemical staining was carried out using a Dako Envision+kit (Dako) with hematoxylin counterstaining. Immunostaining with subclass-matched irrelevant IgG served as a negative control. Apoptotic nuclei were determined by terminal deoxyribonucleotide transferase (TdT)-mediated nick-end labeling (TUNEL) using a commercially available kit (*in situ* apoptosis detection kit, TACS, Trevigen, Gaithersburg, MD, U.S.A.).[19] Specimens stained without TdT enzyme served as negative controls. Counterstaining with hematoxylin was also performed. Tissue fixation, paraffin embedding, and LOX-1 and TF staining procedure were performed in the same condition, respectively.

Classification of Atherosclerotic Lesions The atherosclerotic lesions in WHHLMI rabbits were divided into 4 categories using a classification scheme based on the recommendations of the American Heart Association (AHA)[20,21] by HE and Azan–Mallory staining, as previously described[19]: (**1**) neointimal lesion (Type I—III), (**2**) atheromatous lesion (Type IV), (**3**) fibroatheromatous lesion (Type Va, Vb), (**4**) collagen-rich lesion (Type Vc), as shown in Fig. 1. Neointimal lesions were defined as having adaptive thickening of the intima consisting mainly of smooth muscle cells and few macrophages. Atheromatous lesions consisted of macrophages (foam cells) rich and lipid-rich areas covered with thin fibrous connective tissue, and were considered to be similar to vulnerable lesions in human atherosclerotic plaques. Fibroatheromatous lesions were composed of several macrophages (foam cells) and lipid-rich areas separated by thick layers of fibromuscular connective tissue, which were relatively stable to rupture.[22] Collagen-rich lesions contained predominant collagen components and smooth muscle cells.

Semi-quantitative Analyses Areas (μm^2) occupied by each lesion component were evaluated with a VHX Digital Microscope (Keyence Corp., Osaka, Japan). The vulnerability index, an index of the morphological destabilized characteristics of atherosclerotic lesions in WHHLMI rabbits, was calculated for each atherosclerotic lesion as previously described.[23] The vulnerability index was defined as the ratio of the lipid component area (macrophages+extracellular lipid deposits)/fibromuscular component area (smooth muscle cells+collagen fibers). Collagen-rich fibers and extracellular lipid deposits (extracellular vacuoles and lacunae) were determined with Azan–Mallory staining.[19] The macrophage and smooth muscle cell areas were determined with immunohistochemical staining by use of antibodies RAM11 and 1A4, respectively. LOX-1 and TF expression were assessed as percentages of positively stained areas (% positive). The number of TUNEL-positive cell (apoptotic cell) was counted under the microscope and the TUNEL-positive cell density (number/mm^2) was calculated.

In order to evaluate the detailed expression of LOX-1 and TF in atheromatous and fibroatheromatous lesions, these lesions were subdivided into three subregions; fibromuscular cap, lipid area and boundary regions (Fig. 5I).[21] The intensity of immunohistochemical staining for LOX-1 and TF was examined in each subregion and scored from 0 to 2 (0, negative; 1, equivocal; 2, intense). The evaluation was performed in a blinded fashion four times by two independent observers (S.I. and N.T.) for each specimen.

Statistical Analyses Data are presented as the mean± S.D. Comparisons among lesion types were performed using the Kruskal–Wallis test with *post hoc* analysis by the Scheffe test. Correlation coefficients were assessed with Spearman rank correlation coefficients. Statistical significance was defined as $p<0.05$.

RESULTS

Composition of Atherosclerotic Lesions In the aortic arch and thoracic aorta of 4 WHHLMI rabbits, 191 histopathological features which correspond to the classification criteria (neointimal, atheromatous, fibroatheromatous or collagen-rich lesions) were observed. Thus, the 191 regions were divided into the 4 lesion-categories. Figure 1 shows typical images of four categorized lesion types with HE, Azan–Mallory and immunohistochemical staining (Figs. 1A—H). Fourteen lesions were classified as neointimal lesions showing a thin intimal layer consisting of smooth muscle cells and few macrophages (Fig. 1, left column). Forty-four lesions were classified as atheromatous lesions, consisting of a large

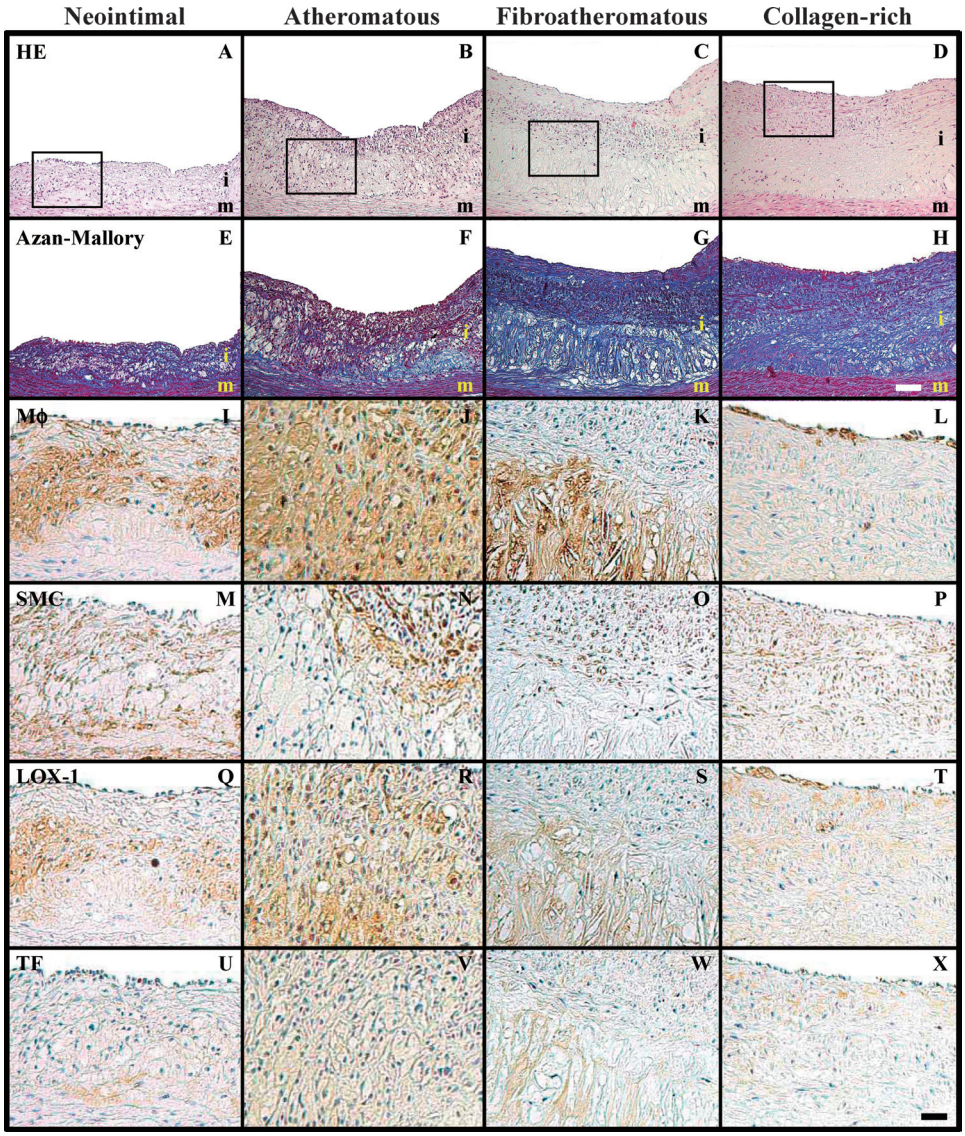

Fig. 1. LOX-1 and TF Expression in 4 Categorized Atherosclerotic Lesions of WHHLMI Rabbits

Atherosclerotic lesions were microscopically classified into four categories as described in Materials and Methods: neointimal lesion (left column), atheromatous lesion (middle-left column), fibroatheromatous lesion (middle-right column) and collagen-rich lesion (right column). HE staining (A—D), Azan–Mallory staining (E—H) and immunohistochemical staining for macrophage staining (Mϕ) (I—L), smooth muscle cell staining (SMC) (M—P), LOX-1 (Q—T) and TF (U—X) are shown. The panels of immunohistochemical staining showed higher magnification images of the area indicated in A—D. Bar=100 μm, magnification ×40 (A—H), Bar=300 μm, magnification ×120 (I—X), m: media, i: intima.

lipid area with abundant macrophages and extracellular lipid deposits, accompanied by a thin fibromuscular cap with relatively few smooth muscle cells scattered in the superficial region (Fig. 1, middle-left column). Fifty-six lesions showed typical characteristics of fibroatheromatous lesions, consisting of few macrophages, less extracellular lipid deposits and a thicker fibromuscular cap (Fig. 1, middle-right column). Seventy-seven lesions were categorized as collagen-rich lesions, consisting of a thick neointimal layer with abundant fibrous connective tissue and smooth muscle cells, while the lipid area was minimal or even absent; collagen-rich lesions (Fig. 1, right column). The atheromatous, fibroatheromatous and collagen-rich lesions were similarly observed in both of the aortic arch and the descending thoracic aorta; however, neointimal lesions were found only in the aortic arch, but not in the descending thoracic aorta. Neither plaque rupture nor thrombi (type VI) were detectable in the aortic arch or the descending thoracic aorta of the rabbits used in the present study.

LOX-1/TF Expression and Apoptotic Events in Atherosclerotic Lesions Prominent LOX-1 expression was found in the macrophage-rich lipid areas of atheromatous and fibroatheromatous lesions (Figs. 1Q—T). LOX-1 expression was also observed in the superficial part of neointimal lesions and collagen-rich lesions. In addition, scattered positive staining for LOX-1 was observed in the neointima of collagen-rich lesions. LOX-1 expression was mainly detected in macrophages and endothelial cells, as well as slight to moderate expression in the smooth muscle cells (Figs. 1I—T). TF expression was prominent in the macrophage-rich lipid areas of atheromatous lesions, and the expression was mainly observed in macrophage foam cells (Figs. 1I—L, U—X). TUNEL-positive cells were extensively observed in the macrophage-rich lipid areas of the atheromatous and fibroatheromatous lesions where a large number of foam cells were accumulated (Figs. 2B, C). Scattered TUNEL-positive

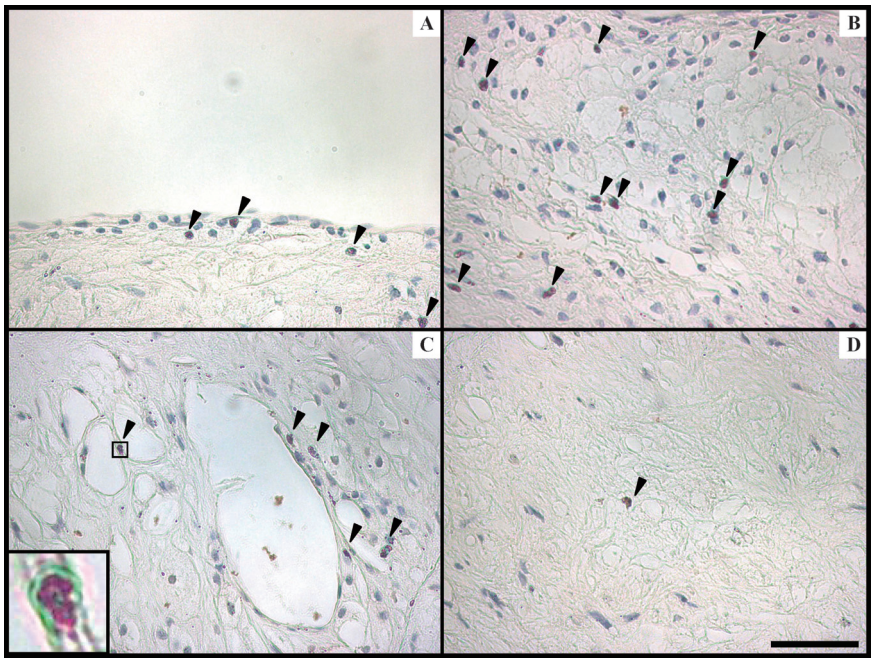

Fig. 2. Detection of TUNEL-Positive Nuclei in 4 Categorized Atherosclerotic Lesions of WHHLMI Rabbits

DNA fragmentation staining by *in situ* end labeling (TUNEL) are shown. Scattered TUNEL-positive nuclei (arrow head) were observed in the intimal region (A), the macrophage-rich lipid area of fibroatheromatous lesion (C) and the collagen layer (D). TUNEL-positive nuclei were mainly observed in the macrophage-rich lipid area of the atheromatous lesion (B). Bar=50 μm, magnification ×300. In panel (C), a higher magnification image of a TUNEL-positive nucleus was indicated (inlet).

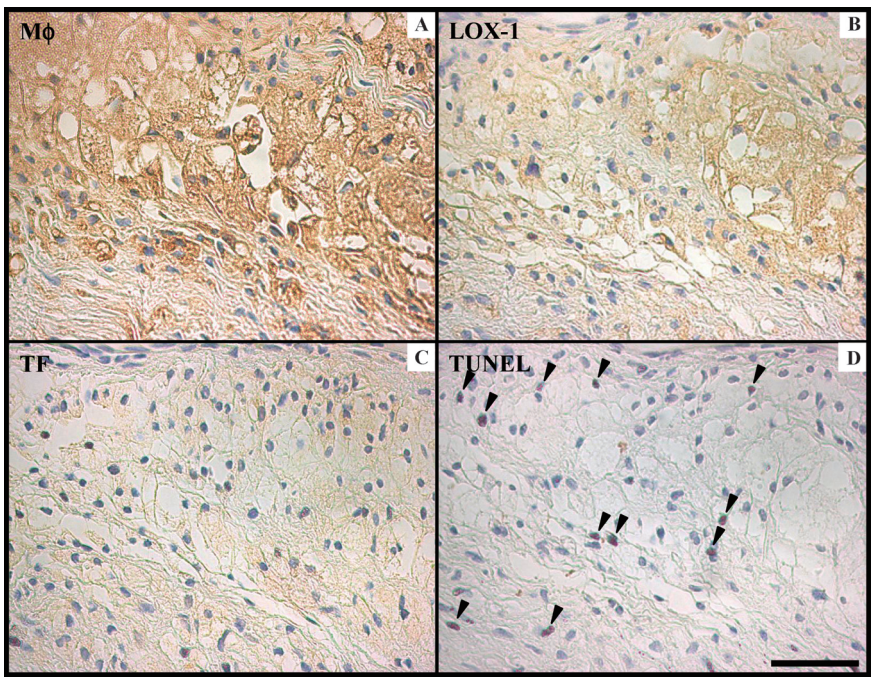

Fig. 3. Typical Microscopic Images of Immunohistochemical Staining for Macrophages (A), LOX-1 (B) and TF (C), and TUNEL Staining (D) for the Macrophage-Rich Area of the Atheromatous Lesion

LOX-1 expression was mainly observed in the macrophage-rich lipid area where TF expression and TUNEL-positive nuclei were prominent. Bar=50 μm, magnifications ×300.

cells were found in the fibromuscular areas of the collagen-rich lesions (Fig. 2D). TUNEL-positive cells in the superficial region were much less frequently seen among all atherosclerotic lesions (Fig. 2A).

Figure 3 shows typical microscopic images of immunostaining and TUNEL for the macrophage-rich lipid area of atheromatous lesions at higher magnifications. LOX-1 expression, TF expression and TUNEL-positive cells were prominently observed in the macrophage-rich lipid areas (Fig. 3).

There was no notable difference in the staining tendency of LOX-1, TF and TUNEL-positive cells between the aortic arch and the descending thoracic aorta. Neither LOX-1, TF, nor TUNEL-positive cells were detectable by immunostain-

ing in negative control sections (data not shown).

Semi-quantitative Analyses of LOX-1/TF Expression and Apoptotic Events in Relation to Plaque Vulnerability Figure 4a shows the vulnerability index calculated for each lesion category classified as described in Materials and Methods. This index was the highest in the atheromatous lesions ($p<0.0001$ *vs.* other lesions), followed in decreasing order by the fibroatheromatous lesions ($p<0.005$ *vs.* neointimal lesions; $p<0.0001$ *vs.* collagen-rich lesions), the neointimal lesions, and the collagen-rich lesions.

LOX-1 expression (% positive) was the highest in the atheromatous lesions ($p<0.05$ *vs.* neointimal lesions; $p<0.0001$ *vs.* fibroatheromatous and collagen-rich lesions, Fig. 4b), followed in decreasing order by the neointimal lesions, the fibroatheromatous lesions, and the collagen-rich lesions. TF expression (% positive) in the atheromatous lesions was also the highest among the four lesion types ($p<0.0001$ *vs.* other lesions, Fig. 4c). DNA nick end-labeling of tissue sections showed that the TUNEL-positive cell density (number/mm^2) was higher in the atheromatous lesions than those in the fibroatheromatous and collagen-rich lesions ($p<0.0001$, Fig. 4d). In the neointimal lesions, the TUNEL-positive cell density was higher than collagen-rich lesions ($p<0.05$).

Localization of LOX-1 and TF Expression within Atherosclerotic Lesions The atheromatous and fibroatheromatous lesions were subdivided into three subregions: fibromuscular cap (C), lipid area (foam cells+extracellular lipid deposits) (L) and boundary regions (B) as illustrated in Fig. 5I. Staining intensity scores for LOX-1 and TF in each subregion of atheromatous and fibroatheromatous lesions were compared. The expression levels of LOX-1 were significantly higher in the lipid area than in the fibromuscular cap and

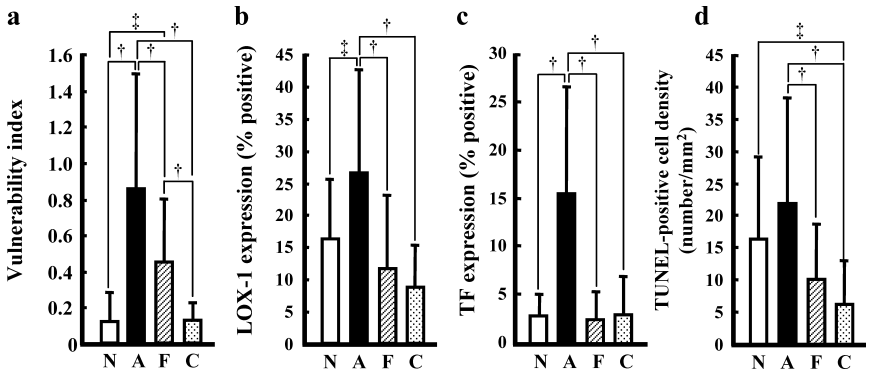

Fig. 4. Vulnerability Index (a), Percentages of Positively Stained Areas (% Positive) for LOX-1 (b) and TF (c), and TUNEL-Positive Cell Density (Number/mm^2) (d) in the Classified Atherosclerotic Lesions

N, A, F and C indicate neointimal, atheromatous, fibroatheromatous and collagen-rich lesions, respectively. Data are represented as the mean±S.D. †$p<0.0001$, ‡$p<0.05$.

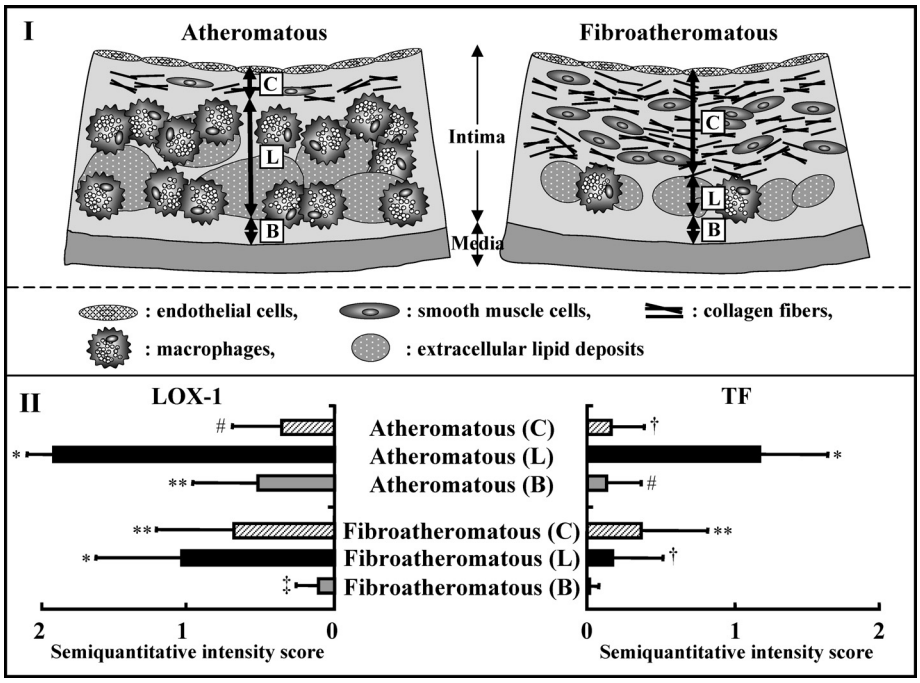

Fig. 5. LOX-1 and TF Expression Scores in Fibromuscular Cap (C), Lipid Area (Foam Cells+Extracellular Lipid) (L), and Boundary (B) Subregions of Atheromatous and Fibroatheromatous Lesions

(I) Schematic illustration of fibromuscular cap (C), lipid area (L) and boundary (B) subregions, which compose atheromatous (left) and fibroatheromatous (right) lesions. (II) Expression scores of LOX-1 (left) and TF (right) in fibromuscular cap (C), lipid area (L) and boundary (B) subregions of 44 atheromatous (upper) and 56 fibroatheromatous (lower) lesions. Data are indicated as the mean±S.D. in each subregion. *$p<0.0001$ *vs.* all other regions. **$p<0.0001$ *vs.* fibroatheromatous (B). #$p<0.0001$ *vs.* fibroatheromatous (C). †$p<0.05$ *vs.* fibroatheromatous (C). ‡$p<0.05$ *vs.* atheromatous (C).

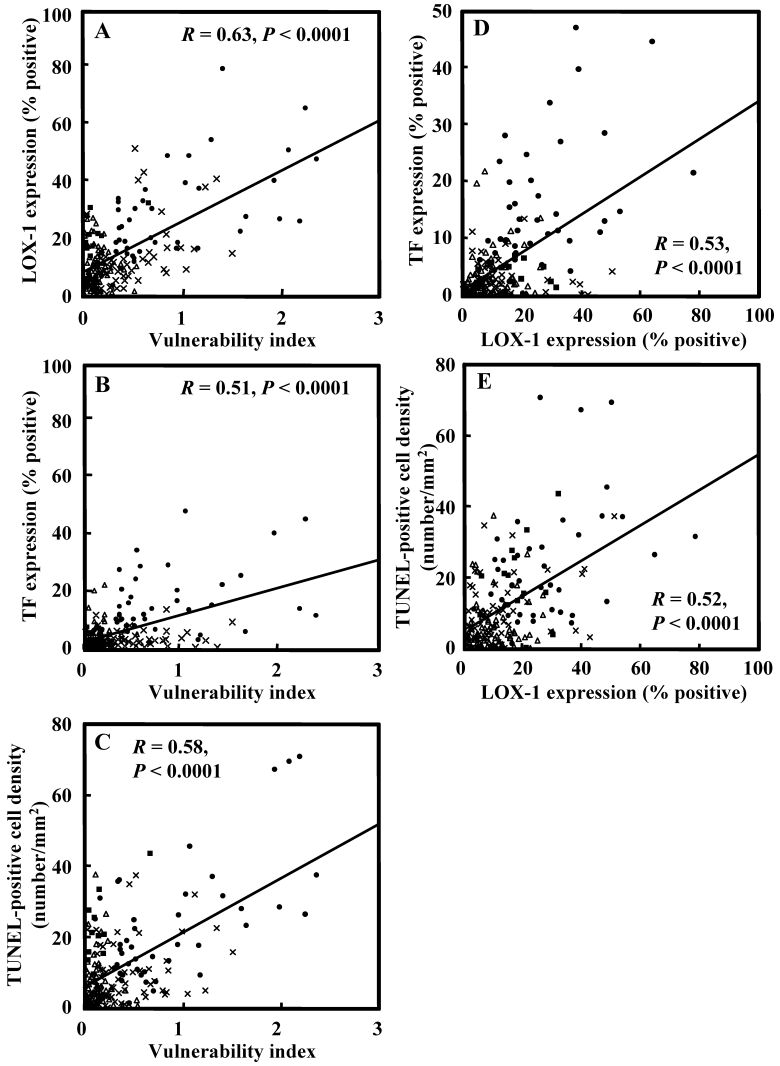

Fig. 6. Analyses of Correlations among LOX-1 and TF Expression (% Positive), and TUNEL-Positive Cell Density (Number/mm^2) and Histological Vulnerability

Regression analyses demonstrated a positive correlation between vulnerability index and LOX-1 expression (r=0.63, p<0.0001) (A), between vulnerability index and TF expression (r=0.51, p<0.0001) (B), as well as between vulnerability index and TUNEL-positive cell density (r=0.58, p<0.0001) (C). In the same manner, a positive correlation between LOX-1 expression and TF expression (r=0.53, p<0.0001), as well as between LOX-1 expression and TUNEL-positive cell density (r=0.52, p<0.0001) are shown in panel (D) and (E), respectively. ■; neointimal, ●; atheromatous, ×; fibroatheromatous, △; collagen-rich lesions.

boundary regions (Fig. 5II). The LOX-1 expression levels were the highest in the lipid area of atheromatous lesions among all subregions of any lesion types (p<0.0001), followed by the lipid area of fibroatheromatous lesions. The TF expression levels were also the highest in the lipid area of atheromatous lesions among all subregions of any lesion types.

Correlation Analyses among LOX-1, TF, Apoptosis and Histological Vulnerability Correlation of the vulnerability index with LOX-1 and TF expression (% positive), and TUNEL-positive cell density (number/mm^2) is shown in Figs. 6A—C. LOX-1 expression was positively correlated with the vulnerability index (r=0.63, p<0.0001, Fig. 6A). Similarly, TF expression and TUNEL-positive cell density showed positive correlation with the vulnerability index (r=0.51, p<0.0001 and r=0.58, p<0.0001, respectively, Figs. 6B, C).

Figures 6D and E show the correlation of LOX-1 expression with TF expression (D) and TUNEL-positive cell density (E). Regression analyses demonstrated positive correlation between LOX-1 and TF expression (r=0.53, p<0.0001, Fig. 6D), as well as between LOX-1 expression and TUNEL-positive cell densities (r=0.52, p<0.0001, Fig. 6E).

DISCUSSION

Our major findings in this study can be summarized as follows: (1) significantly enhanced LOX-1, TF expression and apoptotic events were observed in the atheromatous lesions characteristic of the vulnerable plaque. (2) LOX-1 expression was mainly observed in the macrophage-rich lipid area where TF expression and apoptotic events were prominent. (3) LOX-1 expression levels were positively correlated with morphological vulnerability (vulnerability index), TF expression and apoptotic events. These findings are the first *in vivo* data suggesting the roles of LOX-1 in the processes of plaque destabilization and subsequent thrombus formation.

In the present study, we investigated the LOX-1 expression

in WHHLMI rabbits that developed various atherosclerotic changes, early to advanced lesions, in the same individual. Consequently, we found that the LOX-1 expression was the highest in the atheromatous lesions (type IV) (Fig. 4B), which could mainly be caused by extensive LOX-1 expression in macrophages-rich area (Fig. 1R, 5II left). The current results support our earlier study where we showed a correlation between LOX-1 expression and morphological plaque instability.[16] In addition, Kataoka *et al.* reported LOX-1 expression in macrophages and smooth muscle cells in human advanced atherosclerotic lesions.[24] On the other hand, Chen *et al.* reported that LOX-1 expression was observed in endothelial cells, macrophages and smooth muscle cells in early atherosclerotic lesions.[25] The moderate LOX-1 expression in the neointimal lesions observed in our rabbits was consistent with the results reported by Chen *et al.*

TF has been known as an initiating factor in the coagulation protease cascade. TF expression has been suggested by several studies to be linked with thrombus formation after the plaque rupture. TF expression has been detected within the macrophage-rich areas, but is absent in the fibrous tissue.[26,27] Concordantly, TF expression was extensively shown in the lipid area (Fig. 5II right) of atheromatous lesions (Fig. 4C) in the present study. The spatial distribution of TF and LOX-1 immunostaining in serial sections suggests the colocalization of these antigens in RAM-11-positive macrophage-rich area (Figs. 1, 3A—C, 5). In addition, the TF expression was positively correlated with the LOX-1 expression in the atherosclerotic plaques (Fig. 6D). Thus, our data provide *in vivo* evidence for the correlated expression between LOX-1 and TF. These findings appear to suggest that LOX-1 may be involved in Ox-LDL-induced TF expression in the lipid rich plaques *in vivo*. The CD40/CD40L signaling pathway may be involved in this process, since Ox-LDL-LOX-1 interactions activate the CD40/CD40L signaling pathway,[13,14] which thereby induces TF expression.[15] Thus, LOX-1 may play a key role in TF expression, and thereby thrombus formation *in vivo*. In addition, previous *in vitro* studies have also reported that expression of TF and LOX-1 are mediated by nuclear factor-κB (NF-κB) activation.[28—30] The proinflammatory transcription factor, NF-κB, has been known to mediate transcription of a wide variety of genes induced by proinflammatory simuli, including IL-1α, IL-1β, TNF-α, and Ox-LDL.[31] Therefore, coordinated expression of TF and LOX-1 in the macrophage-rich lipid area of the atheromatous lesions may be regulated by NF-κB.

Apoptosis is one of the causative factors in the formation of vulnerable atherosclerotic plaques. Apoptosis of foam cells and macrophages is thought to promote plaque vulnerability by causing the accumulation of an acellular (cell-poor) lipid core.[4] In the present study, LOX-1 expression was prominent in the macrophage-rich lipid area where TUNEL-positive cells were mainly observed (Figs. 3A—D). When quantified by histomorphometry, the significant correlation between LOX-1 expression and apoptotic events in the atherosclerotic lesions was observed (Fig. 6E). On the other hand, the translocation of phosphatidylserine to the cell surface that occurred during apoptotic cell death was reportedly correlated to a high TF expression.[26,32] Concordantly, we detected marked TF expression in close proximity to apoptotic foam cells within the macrophage-rich lipid area (Figs. 3C, D). Taken together, a potential *in vivo* interaction among LOX-1, TF and apoptotic events may be proposed. LOX-1 may promote TF expression not only *via* the CD40/CD40L signaling pathway, but also the apoptosis of foam cells in the macrophage-rich lipid area of atheromatous lesions. Further studies on the LOX-1-dependent activation of caspase-9 and caspase-3, and/or the regulation of Bcl-2 and c-IAP-1[11] would clarify the potential linkage.

It is also reported that the apoptosis of smooth muscle cells could weaken the fibrous cap *via* a decrease in the synthesis of the extracellular matrix.[5] Previous studies suggested that LOX-1 expression may also play a role in the development of apoptosis in smooth muscle cells.[10,11,24] In the present study, however, TUNEL-positive nuclei were mainly observed in macrophage-rich lipid area where LOX-1 expression is abundant using a rabbit hyperlipidemic model. Several previous studies have indicated that Ox-LDL-LOX-1 interaction in macrophages promotes MMPs involved in the degradation of extracellular matrix proteins,[12] which is consistent with our current and previous results in WHHLMI rabbits.[16]

It is of great importance to discuss the animal model used in the present study (WHHLMI rabbits). Unfortunately, animal models suitable for studying the spontaneous rupture of unstable plaques have yet to be established. However, it has been reported that hemorrhage, plaque rupture and thrombosis (type VI) are occasionally observed in coronary lesions of WHHLMI rabbits, although the atherosclerotic plaques are not prone to rupture.[33] In fact, the pathological features of atheromatous lesions observed in our rabbits were similar to those of human unstable plaques.[17,18,34,35] Thus, the use of WHHLMI rabbits could be effective for investigating causative factors in the destabilization of atherosclerotic plaques and help translate laboratory observations to the clinical setting.

CONCLUSION

The present data from the atherosclerotic specimens of WHHLMI rabbits demonstrate a pathobiological link among LOX-1, TF, apoptotic events and the vulnerability of atherosclerotic plaques, suggesting that the vulnerability and thrombotic activity of atherosclerotic lesions may result from a LOX-1 signal linkage. The present findings should help understand the pathophysiology of atherosclerosis, leading to the development of new therapeutic and diagnostic (imaging) agents of atherosclerosis.

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